

FIBRIN CITRULLINE DERIVATIVES AND THEIR USE FOR  
DIAGNOSING OR TREATING RHEUMATOID ARTHRITIS

The present invention relates to citrullinated  
5 derivatives of fibrin and to their uses in diagnosing  
and treating rheumatoid arthritis.

Rheumatoid arthritis (hereinafter abbreviated to "RA")  
is the most common of the forms of chronic inflammatory  
10 rheumatism. It is an autoimmune disease; the serum of  
affected patients contains autoantibodies, some of  
which are specific and may constitute a marker for this  
disease, allowing it to be diagnosed even at early  
stages.

15 Prior studies by the team of the inventors have shown  
that these antibodies recognize different molecular  
forms of the (pro)filaggrin family (for review, cf. for  
example SERRE and VINCENT, In: Autoantibodies, PETER  
20 and SHOENFIELD Eds, Elsevier Science Publishers, 271-  
276, 1996). These antibodies have, for this reason,  
been named: "antifilaggrin autoantibodies (AFAs)".  
Application EP 0 511 116 describes the purification and  
characterization of antigens of the filaggrin family,  
25 recognized by these antibodies, and their use for  
diagnosing rheumatoid arthritis.

The inventors have shown that the epitopes recognized  
by the AFAs are carried by regions of the filaggrin  
30 molecule, in which at least some of the arginines are  
deiminated and thus transformed into citrulline;  
citrullinated peptides specifically recognized by AFAs  
have thus been obtained from the main immunoreactive  
regions of filaggrin. These peptides, and their use for  
35 diagnosing RA, are the subject of Application  
PCT/FR97/01541 and of Application PCT/FR98/02899 in the  
name of BIOMERIEUX. The inventors' observations  
concerning the role of citrulline residues in the  
reactivity of filaggrin with RA-specific autoantibodies

have subsequently been confirmed by other researchers [SCHELLEKENS et al., Arthritis Rheum., 40, no. 9 supplement, p. S276, summary 1471 (1997); VISSER et al., Arthritis Rheum., 40, no. 9 supplement, p. S289, summary 1551 (1997)].

The inventors have also shown that AFAs represent a considerable proportion of the interstitial immunoglobulins of synovial rheumatoid tissues and that they are synthesized locally by specific plasmocytes present in these tissues, which confirms the hypothesis that they are involved in the autoimmune response associated with RA. The use of filaggrin, or of citrullinated peptides derived therefrom, to neutralize this autoimmune response is the subject of Application PCT/FR98/02900 in the name of UNIVERSITÉ PAUL SABATIER [Paul Sabatier University] (TOULOUSE III).

However, the involvement of filaggrin as an immunogen or as a target antigen in the autoimmune response associated with RA has never been noted. The true antigen involved in this response remains to be identified.

The inventors have now succeeded in characterizing this antigen and have thus shown that it is composed of citrullinated derivatives of the  $\alpha$ - and/or  $\beta$ -chains of fibrin.

A subject of the present invention is a citrullinated polypeptide derived from all or part of the sequence of the  $\alpha$ -chain or of the  $\beta$ -chain of a vertebrate fibrin, by substitution of at least one arginine residue with a citrulline residue.

Preferably, a polypeptide in accordance with the invention comprises at least 5 consecutive amino acids and advantageously at least 10 consecutive amino acids,

including at least one citrulline, of the sequence of the  $\alpha$ -chain or of the  $\beta$ -chain of a mammalian fibrin. Advantageously, said vertebrate fibrin is a mammalian fibrin, preferably a human fibrin.

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Citrullinated polypeptides in accordance with the invention may, for example, be obtained from natural, recombinant or synthetic fibrin or fibrinogen, or from fragments thereof, comprising at least one arginine residue, by the action of peptidyl arginine deiminase (PAD); they may also be obtained by peptide synthesis, directly incorporating one or more citrulline residues into the synthesized peptide.

15 Citrullinated polypeptides in accordance with the invention may also be pseudopeptides having the same three-dimensional structure, and therefore the same immunological reactivity, as the citrullinated polypeptides derived from the  $\alpha$ - or  $\beta$ -chains of fibrin, or from fragments thereof, mentioned above. They may, for example, be pseudopeptides of the retro type, in which L-amino acids are linked together according to a reverse sequence of that of the peptide to be reproduced, or pseudopeptides of the retro-inverso type, consisting of D-series amino acids (instead of the L-series amino acids of natural peptides) linked together according to a reverse sequence of that of the peptide to be reproduced, or alternatively pseudopeptides containing a  $\text{CH}_2\text{-NH}$  bond in place of a CO-NH peptide bond. Pseudopeptides of these various types are, for example, described by BENKIRANE et al. [J. Biol. Chem., 270, p. 11921-11926, (1995); J. Biol. Chem., 271, p. 33218-33224, (1996)]; BRIAND et al. [(J. Biol. Chem., 270, p. 20686-20691, (1995); GUICHARD et al. [J. Biol. Chem., 270, p. 26057-26059, (1995)].

A subject of the present invention is also the use of the polypeptides in accordance with the invention, as defined above, for diagnosing RA, *in vitro*.

5 The present invention in particular encompasses antigenic compositions for diagnosing the presence of RA-specific autoantibodies in a biological sample, which compositions are characterized in that they contain at least one polypeptide in accordance with the  
10 invention, optionally labeled with and/or conjugated to a carrier molecule.

A subject of the present invention is also a method for detecting RA-specific autoantibodies of the G class in  
15 a biological sample, which method is characterized in that it comprises:

- bringing said biological sample into contact with at least one polypeptide in accordance with the invention,  
20 as defined above, under conditions which allow the formation of an antigen/antibody complex with the RA-specific autoantibodies possibly present;

- detecting, by any suitable means, the  
25 antigen/antibody complex possibly formed.

This detection method may be carried out using a kit comprising at least one antigen according to the invention, and also buffers and reagents suitable for  
30 constituting a reaction medium which allows the formation of an antigen/antibody complex, and/or means for detecting said antigen/antibody complex.

Said kit may also comprise, where appropriate,  
35 reference samples, such as one or more negative serum (sera) and one or more positive serum (sera).

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[illegible]

The present invention will be more clearly understood using the additional description which follows, which refers to the identification of deiminated forms of the  
5  $\alpha$ -chain or  $\beta$ -chain of human fibrin in rheumatoid tissues, and to the use of deiminated fibrinogen for detecting the presence of AFAs in serum samples.

**EXAMPLE 1: PURIFICATION AND CHARACTERIZATION OF  
10 ANTIGENIC PROTEINS RECOGNIZED BY AFAs IN RHEUMATOID  
SYNOVIAL TISSUES**

**1) Analysis of rheumatoid synovial tissues**

**Materials and methods:**

15 The synovial tissue samples used for the protein extractions were taken from patients suffering from rheumatoid arthritis, during a synovectomy or an arthroplasty of the wrist or knee, and all correspond  
20 to tissue fragments which are the seat of conventional histological rheumatoid synovitis lesions. They are conserved by freezing in isopentane cooled with liquid nitrogen.

25 Synovial tissue fragments originating from four patients were extracted sequentially, in a low ionic strength buffer, a urea buffer and in a urea/DTT buffer, successively.

30 *Preparation of synovial extracts*

The extraction was carried out using an Ultra-Turrax homogenizer (T25 basic, IKA Labortechnik, Staufen, Germany) with a volume of 6 ml of buffer per gram of  
35 tissue.

The following buffers were used at a temperature of 0°C:  
40 mM Tris-HCl, pH 7.4, containing 150 mM of NaCl [low

ionic strength buffer]; 40 mM Tris-HCl, pH 7.4, containing 8M urea deionized on an ion exchange resin (AG 501-X8, Biorad, Hercules, CA) [urea buffer]; 40 mM Tris-HCl, pH 7.4, containing 8M deionized urea and 50 mM dithiothreitol (DTT), (Sigma) [urea/DTT buffer]. All the buffers were supplemented with 20 mM EDTA, 0.02% sodium azide, 2 µg/ml aprotinin, 10 mM N-ethylmaleimide and 1 mM phenylmethylsulfonyl fluoride (Sigma, Saint Louis, MI). After each extraction, the homogenates were centrifuged for 20 minutes at 15,000 g, at the temperature of 4°C. The urea buffer and urea/DTT buffer extracts were dialyzed against water before being analyzed by electrophoresis and by immunotransfer.

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#### *Electrophoresis and immunodetection*

The synovial proteins of the various extracts were separated by electrophoresis on a 10% polyacrylamide gel in denaturing SDS buffer (SDS-PAGE), and were then electrotransferred onto reinforced nitrocellulose membranes (Hybond-<sup>TM</sup>C extra, Amersham, Little Chalfont, UK).

25 The membranes were immunodetected with the following antibody preparations; AFA-positive or AFA-negative rheumatoid human sera; non-rheumatoid control human sera derived from patients suffering from other forms of inflammatory rheumatism or from healthy individuals (1/100); purified fractions of AFAs (10 µg/ml); mouse monoclonal antibody directed against human fibrin and fibrinogen (5 µg/ml); two sheep antisera directed, respectively, against recombinant α- and γ-chains of human fibrinogen (1/1000) (Cambio, Cambridge, UK); a rabbit antiserum directed against the recombinant β-chain of human fibrinogen (1/200000) (Cambio).

The human sera used are derived from 95 patients suffering from rheumatoid arthritis (RA), perfectly characterized from a clinical and biological point of view according to the criteria of the American College of Rheumatology, from 24 patients suffering from non-rheumatoid inflammatory rheumatism or from non-inflammatory pathological conditions (control sera) and from 10 healthy individuals. The semi-quantitative titration of the antifilaggrin antibodies (AFAs) in the sera was carried out by indirect immunofluorescence on cryosections of rat esophageal epithelium and by immunotransfer on epidermal extracts enriched in filaggrin acid variant, according to previously published protocols [VINCENT et al., Ann. Rheum. Dis., 48, 712-722 (1989); VINCENT et al., J. Rheumatol., 25, 838-846 (1998)]. The "AFA-positive" sera are those which exhibit AFAs at significant titers after detection using both methods, and the "AFA-negative" sera are those which do not exhibit detectable AFAs by either of the two methods.

The AFAs were purified by affinity chromatography on the epidermal filaggrin acid variant, according to the protocol described by GIRBAL-NEUHAUSER et al. (J. Immunol., 162, 585-594 (1999), using 45 rheumatoid sera having a high AFA titer. The purified antibody fractions were pooled.

Peroxidase-conjugated secondary molecular probes were used for detecting all the primary antibodies: protein A (Sigma), sheep antibodies directed against mouse IgGs (Biosys, Compiègne, France), goat Fab fragments directed against rabbit IgGs (Biosys) and rabbit F(ab')<sub>2</sub> fragments directed against sheep IgGs (Southern Biotech. Inc), for detecting, respectively, human, murine, rabbit and sheep IgGs. The peroxidase activity was visualized using the ECL<sup>TM</sup> detection system



(Amersham International, Aylesbury, UK), according to the protocol provided by the manufacturer.

### Results

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Specific reactivity with the purified AFAs and the AFA-positive rheumatoid sera was observed only in the extract produced in urea/DTT buffer.

10 The results are illustrated by figure 1:

Legend to figure 1:

- AFAP = purified AFAs;
- RA sera = rheumatoid sera:
  - 15 \* AFA+ = AFA-positive;
  - \* AFA- = AFA-negative;
- control sera = sera derived from patients suffering from forms of inflammatory rheumatism other than RA, or from healthy donors.

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These results show that the specific reactivity with the purified AFAs and the AFA-positive rheumatoid sera relates to two protein bands of apparent molecular weight of approximately 64 kD to approximately 78 kD (w64-78) and of approximately 55 kD to approximately 61 kD (w55-61), respectively. These protein bands were not detected by the AFA-negative sera, regardless of whether they originate from patients suffering from RA or from other forms of inflammatory rheumatism, or are  
25  
30 derived from healthy donors.

The presence of these proteins specifically recognized by the purified AFAs and the AFA-positive rheumatoid sera was observed in the urea/DTT extracts of synovial  
35 tissues derived from the 4 rheumatoid patients studied.

In total, 48 AFA-positive rheumatoid sera were tested by immunotransfer on at least one synovial urea/DTT

extract. Among the sera, 40 recognized w64-78, 39 recognized w55-61, 37 recognized both w64-78 and w55-61, 3 recognized only w64-78 and 2 recognised only w55-61.

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Thirteen AFA-negative rheumatoid sera were tested by immunotransfer on at least one urea/DTT extract of synovial tissue; none of these sera recognized either w64-78 or w55-61.

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Ten sera derived from healthy donors and 5 sera derived from patients suffering from other forms of inflammatory rheumatism were also tested by immunotransfer on at least one synovial urea/DTT extract; none of these sera recognized either w64-78 or w55-61.

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## 2) Characterization of the w64-78 and w55-61 antigenic proteins

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The proteins of the urea/DTT buffer extract of the synovial tissue of one of the patients suffering from RA were precipitated with 4 volumes of glacial acetone and then redissolved in the urea/DTT buffer at a concentration 15 times higher than their initial concentration.

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The proteins of the concentrated extract were separated by two-dimensional electrophoresis, by isoelectrofocussing followed by SDS-PAGE.

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A two-dimensional electrophoretic separation was carried out in the PhastSystem™ (Pharmacia). The first electrophoretic separation was performed on PhastGel™ isoelectrofocussing (IEF) gels which, beforehand, had been washed, dried and rehydrated in a deionized buffer containing 8 M urea, 0.5% Nonidet P-40 and ampholytes creating a pH gradient of 3 to 10 (Pharmacia). The

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second dimension was performed by SDS-PAGE on 7.5% polyacrylamide gels.

The proteins were then electrotransferred onto  
5 polyvinyl difluoride (PVDF) membranes (ProBlott™  
membranes, Applied Biosystems, Foster City, CA), in  
50 mM Tris and 50 mM of boric acid. The membranes were  
finally stained with an aqueous solution of amido black  
at 0.1%, of acetic acid at 1% and of methanol at 45%,  
10 or immunodetected with rheumatoid sera according to the  
protocol described in 1) above.

Figure 2 illustrates the profiles obtained after  
electrotransfer onto a PVDF membrane and:

- 15
- a) staining with amido black; or
  - b) immunodetection with an AFA-positive rheumatoid  
serum; or
  - c) immunodetection with an AFA-negative rheumatoid  
20 serum.

Legend to figure 2:

- Amido Black = staining with amido black;
- AFA+ = immunodetection with an AFA-positive  
25 rheumatoid serum;
- AFA- = immunodetection with an AFA-negative  
rheumatoid serum.

After staining with amido black, the presence of two  
30 major proteins, with an apparent molecular weight of  
64-78 kD and 55-61 kD and pI of approximately 5.85 to  
approximately 8.45, is observed.

These proteins are immunodetected with the AFA-positive  
35 rheumatoid sera but not with the AFA-negative  
rheumatoid sera.

Using identical transfers onto a PVDF membrane after two-dimensional electrophoresis, membrane fragments corresponding to the center of each immunoreactive zone were excised and then subjected to amino-terminal sequencing in an Applied Biosystems sequencer (494A or 473A), according to the method recommended by the manufacturer.

10 The sequence gly-pro-arg-val-val-glu-arg-his-gln-ser-ala was obtained from the membrane fragment corresponding to the w64-78 antigen. This sequence is strictly identical to the sequence 36-46 of the product of the human fibrinogen  $\alpha$ -chain precursor gene. When membrane fragments corresponding to the right or left ends of the w64-78 immunoreactive zone were excised and then each subjected to three cycles of amino-terminal sequencing, gly-pro-arg sequences were found each time, indicating that the entire p64-78 immunoreactive zone has the same amino-terminal end.

20 The sequence gly-his-arg-pro-leu-asp-lys-lys-arg was obtained from the membrane fragment corresponding to the center of the immunoreactive zone corresponding to the w55-61 antigen. This sequence is strictly identical to the sequence 45-54 of the product of the human fibrinogen  $\beta$ -chain precursor gene. When a membrane fragment corresponding to the left end of the w55-61 immunoreactive zone was excised and then subjected to two cycles of amino-terminal sequencing, the gly-his sequence was found. When a membrane fragment corresponding to the right end of the w55-61 immunoreactive zone was excised and then subjected to six cycles of amino-terminal sequencing, the gly-his-arg-pro-leu-asp sequence and the gly-pro-arg-val-val-glu sequence were found. This indicates that the entire w55-61 immunoreactive zone has the same amino-terminal end and that it partially co-migrates with the w64-78 antigen.

The amino-terminal ends of the w64-78 and w55-61 antigenic proteins correspond, respectively, to the amino-terminal ends of the  $\alpha$ - and  $\beta$ -chains of human fibrinogen after respective cleavage, by thrombin, of fibrinopeptides A and B. The amino-terminal ends of the w64-78 and w55-61 antigenic proteins are therefore identical, respectively, to that of the  $\alpha$ -chain and to that of the  $\beta$ -chain of human fibrin.

The apparent molecular weights of the w64-78 and w55-61 antigens are compatible with the respective theoretical molecular weight values for the  $\alpha$ -chain and for the  $\beta$ -chain of human fibrin.

The identity of the w64-78 antigen and of the  $\alpha$ -chain of fibrin, on the one hand, and that of the w55-61 antigen and of the  $\beta$ -chain of fibrin, on the other hand, were confirmed by analyzing the reactivity of antifibrin(ogen) antibodies with respect to these antigens. By immunotransfer, using an extract of synovial tissue prepared in urea/DTT, the "311" mouse monoclonal antibody, which recognizes the three chains  $\alpha$ ,  $\beta$  and weakly,  $\gamma$  of human fibrinogen and fibrin, is mainly reactive with respect to the w64-78 and w55-61 antigens. Similarly, two antisera, one from sheep and the other from rabbit, directed, respectively, against recombinant  $\alpha$ - and  $\beta$ -chains of fibrinogen, recognized mainly a protein which co-migrates with the w64-78 antigen and a protein which co-migrates with the w55-61 antigen, respectively.

#### **EXAMPLE 2: REACTIVITY OF RHEUMATOID SERA AND OF PURIFIED AFAs WITH DEIMINATED FIBRINOGEN IN VITRO**

The reactivity with respect to deiminated and nondeiminated fibrinogen was studied by immunotransfer. The following were used: the purified AFA fractions,

AFA-positive rheumatoid sera of decreasing titer, 10  
AFA-negative rheumatoid sera and 19 AFA-negative sera  
derived from patients suffering from forms of  
inflammatory or non-inflammatory rheumatism (AFA titers  
5 determined by immunotransfer on epidermal extracts  
enriched in filaggrin acid variant).

The results are illustrated by Figure 3A in the case of  
nondeiminated fibrinogen and by Figure 3B in the case  
10 of deiminated fibrinogen.

Legend to Figure 3:

Figure 3A: non deiminated purified human  
15 fibrinogen;

- 311 = antifibrinogen monoclonal antibody 311;
- control sera = sera derived from patients  
suffering from forms of inflammatory rheumatism  
other than RA, or from healthy donors;
- 20 - RA sera = rheumatoid sera;
  - \* AFA+ = AFA-positive;
  - \* AFA- = AFA-negative;

Figure 3B: purified human fibrinogen deiminated with a  
PAD;

- 25 - 311 = antifibrinogen monoclonal antibody 311;
- C1 = sheep antibody directed against mouse IgGs;
- C2 = sheep antibody directed against protein A;
- control sera = sera derived from patients  
suffering from forms of inflammatory rheumatism  
30 other than RA, or from healthy donors;
- RA sera = rheumatoid sera;
  - \* AFA+ = AFA-positive;
  - \* AFA- = AFA-negative;

35 Nondeiminated fibrinogen

After separation by SDS-PAGE, under the conditions  
described in example 1 above, the nondeiminated

fibrinogen is composed of 3 polypeptides having respective apparent molecular weights 48 kDa, 58 kDa and 69 kDa, corresponding to the expected apparent molecular masses of the  $\alpha$ -,  $\beta$ - and  $\gamma$ -polypeptide chains making up the protein (results not given). The "311" antifibrinogen monoclonal antibody strongly recognizes the  $\alpha$ - and  $\beta$ -polypeptide chains and very weakly the  $\gamma$ -polypeptide chain (Figure 3A).

Antisera specific for each of the  $\alpha$ -,  $\beta$ - and  $\gamma$ -chains of fibrinogen also showed reactivity with respect to the chain against which they were respectively directed (results not shown).

15

#### Deimination of the fibrinogen

A peptidyl arginine deiminase (PAD) purified from rabbit skeletal muscle (Sigma, St. Louis, MO) was used. The human fibrinogen (Calbiochem, San Diego, CA) was incubated at the concentration of 0.86 mg/ml, in the presence or absence of PAD (7 U/mg of protein) for 2 h at 50°C, in 0.1 M Tris-HCl buffer, pH 7.4, containing 10 mM of  $\text{CaCl}_2$  and 5 mM of DTT. These conditions are those which previously made it possible to generate the epitopes on a human recombinant filaggrin, recognized by AFAs [GIRBAL-NEUHAUSER et al., J. Immunol., 162, 585-594 (1999)]. The deimination was then stopped by adding 2% of SDS and heating at 100°C for 3 min.

After deimination for 2 hours, the electrophoretic mobility by SDS-PAGE of the two  $\alpha$ - and  $\beta$ -polypeptides became modified and that of the  $\gamma$ -polypeptide remained unchanged. Specifically, the protein corresponding to the  $\alpha$ -chain then appeared in the form of a diffuse band of 82 to 95 kDa and was immunodetected by both the

"311" antifibrinogen monoclonal antibody (figure 3B) and the antiserum directed against the  $\alpha$ -chain of fibrinogen (results not shown).

5 The protein corresponding to the  $\beta$ -chain appeared in the form of a well-defined doublet with the molecular weight of 458 kD for the lower band and 60 kD for the upper band, which was not recognized by the "311" antifibrinogen monoclonal antibody (figure 3B) but was  
10 immunodetected by the rabbit antiserum directed against the recombinant  $\beta$ -chain of human fibrinogen (results not shown).

No reactivity for the  $\alpha$ -chain or for the  $\beta$ -chain is  
15 observed with the C1 and C2 antibodies.

#### Reactivity of the sera

20 The reactivity of the sera with respect to the  $\alpha$ - and  $\beta$ -chains of nondeiminated fibrinogen proved to be zero or very weak and concerned only a few sera rarely occurring, belonging to no particular subgroup.

25 On the other hand, after deimination, the polypeptides corresponding to the deiminated  $\alpha$ - and  $\beta$ -chains react strongly with the purified AFAs (results not shown) and with all of the 37 AFA-positive rheumatoid sera (with  
30 the exception of that which has the lowest AFA titer). Moreover, 6 AFA-negative rheumatoid sera out of 10 also clearly recognized the deiminated  $\alpha$ - or  $\beta$ -polypeptides:  
2 immunodetected the  $\alpha$ -polypeptide and the  $\beta$ -polypeptide doublet, 3 others only detected the  
35  $\beta$ -polypeptide doublet, and only 1 immunodetected exclusively the  $\alpha$ -polypeptide. On the other hand, with the exception of a serum derived from a patient suffering from Sjögren's syndrome, which was reactive



on the  $\beta$ -polypeptide doublet, none of the control sera immunodetected the deiminated fibrinogen.

20305030607080901001101201301401501601701801902002102202302402502602702802903003103203303403503603703803904004104204304404504604704804905005105205305405505605705805906006106206306406506606706806907007107207307407507607707807908008108208308408508608708808909009109209309409509609709809901000

The affinity of the AFA-positive rheumatoid sera with respect to the two deiminated  $\alpha$ - and  $\beta$ -polypeptides proved to be slightly variable from one serum to the other. Thus, 6 sera, while strongly detecting the  $\beta$ -polypeptide, only very weakly recognized the  $\alpha$ -polypeptide. Similarly, 3 sera, highly reactive with respect to the  $\alpha$ -polypeptide, did not detect the deiminated  $\beta$ -polypeptide. Moreover, the intensity of labeling of the two polypeptides appears, overall, to be proportional to the AFA titer of the sera. It should be noted that the sera reactive on the deiminated  $\alpha$  and  $\beta$ -polypeptides of fibrinogen were also reactive with respect to high molecular weight (greater than 200 kD) polypeptides generated during the deimination of the fibrinogen. These polypeptides which clearly react with the antifibrinogen antibodies are very probably fibrinogen chain aggregates.

In conclusion, recognition of the  $\alpha$ - and  $\beta$ -polypeptides of fibrinogen by rheumatoid sera is not only entirely dependent on their deimination, since the nondeiminated polypeptides are never recognized, but it is also clearly linked to the antifilaggrin reactivity of these sera. It should be noted that these deiminated polypeptides make it possible to detect with great sensitivity the AFAs present in rheumatoid sera.

These results clearly demonstrate that the antigenic targets of the ASAs in rheumatoid synovial joints are deiminated forms of the  $\alpha$ -chain and of the  $\beta$ -chain of human fibrin.

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20305030607080901001101201301401501601701801902002102202302402502602702802903003103203303403503603703803904004104204304404504604704804905005105205305405505605705805906006106206306406506606706806907007107207307407507607707807908008108208308408508608708808909009109209309409509609709809901000

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